A CONTRIBUTION TO THE BACTERIOLOGY OF DIPHTHERIA.*

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Interest in the bacteriology of diphtheria has been directed to the study of the identity of Hofmann's bacillus and the group of "pseudo-diphtheria bacilli," and their relation to the true diphtheria bacillus; to study of methods and means whereby a virulent bacillus may be differentiated from the non-virulent in practical culture work; and to the study of the question of "carriers," from both a bacteriological and public health point of view.

Ever since the discovery of Hofmann's bacillus efforts have been made to establish its relation to the true diphtheria bacillus. Various culture media, differential stains, and passage through various animals have been tried in the endeavor to make it virulent or change its morphology. Similar efforts have been made to convert a typical diphtheria bacillus into the Hofmann type. The results reported have not been uniform, although present indications point to the fact that the Hofmann bacillus possesses such characteristics as to justify its recognition as a separate entity. In my paper on "A Study of Diphtheria Bacilli with Special Reference to Complement-Fixation Reactions,"[1] however, it has been shown by complement-fixation tests with homologous antigens and immune sera of different types of diphtheria bacilli from varied clinical sources, including a true Hofmann bacillus, that all of these bacilli are closely related and that the Hofmann bacillus belongs to the group of diphtheria bacilli, but that as a result of various influences the latter has been changed in its chemical reactions, structure, and virulence so as to give it constant and more or less new characteristic features which are transmitted through a number of generations. Between this decided example of "mutation" and the parent or true diphtheria bacilli are many

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bacilli partaking of some of the features of both. Hence the development of the large indefinite "group" vaguely termed "pseudo-diphtheria" bacilli.

The question of bacillus "carriers" is especially troublesome when one is investigating an epidemic or endeavoring to control diphtheria infection by extensive culture work. To this day the great number of practitioners do not understand why a patient who is apparently fully recovered may still be dangerous to others. In practice it is not possible to isolate every person found, as a result of extensive culture work, to be harboring diphtheria-like bacilli. The "carrier" of virulent diphtheria bacilli is probably immune because of the presence of antitoxin in his blood, or the bacilli may be regarded as examples of "mutation," in that while they have at one time caused disease in the host they have now become adapted to their environment and are harmless to him but not to others.

While it is possible to increase the virulence of an organism by repeated "passage" through the lower animals, it has not definitely been proved possible to give virulence to a known non-virulent organism; in diphtheria culture work we are constantly encountering these non-virulent bacilli. Hence any method enabling the bacteriologist to differentiate between virulent and non-virulent bacilli with a reasonable degree of accuracy and without resorting to expensive and prolonged animal inoculation and acid-production tests would be of great value in the proper management of diphtheria. Largely with this end in view a morphological classification was proposed by Wesbrook, Wilson, and McDaniel, wherein the various bacilli were regarded as dangerous, doubtful, or negligible according to their size and structure. According to this plan differentiation could be made within 24 hours after taking the culture. The value and limitations of the classification can be determined only after extensive comparative bacteriological and clinical studies with a large number of animal inoculation tests for virulence.

The object of this study is therefore as follows:

1. To investigate the relation of types of diphtheria bacilli to clinical diphtheria, with special attention to the value of a mor-

phological classification in bacteriological diagnosis and management of diphtheria.

2. To investigate animal inoculation tests, especially as concerns their value in the management of prolonged positive culture and "carrier" cases.

3. To investigate the value of acid-production tests in differentiating members of the diphtheria group of bacilli.

4. To give practical discussion of the bacteriological diagnosis and management of diphtheria.

TYPES OF BACILLI.

According to the morphological classification of Wesbrook, Wilson, and McDaniel, the various kinds of diphtheria bacilli may be divided into three main groups: granular, barred, and solid bacilli. Each of these groups is subdivided into types according to the size and shape of bacilli, the number and distribution of granules, number of bars, etc. In practical work the routine use of such a classification presents a few difficulties. Many cultures show bacilli of different groups side by side or different types of the same group, so that it is frequently difficult to classify. The differences between some of the types are often so slight as to require high magnification and great care in differentiating. Differences in the length of incubation of cultures and temperature of incubator will frequently alter the results. Thus after short incubation solid types predominate which later may become granular; an incubator temperature over 37° or 38° C. results in the growth of short forms difficult to recognize and classify. Cultures should be incubated for at least 18 hours at 35° C. Ordinary Loeffler's methylene blue is the best routine stain.

The predominating type of organism was recorded in cultures of 253 cases of diphtheria from early in the infection until two successive negative cultures were obtained. In the primary cultures the following types predominated:

A. Tonsillar Diphtheria		186 cases	
1. Granular bacilli	Types AA, B, and C (mostly C)	83.8	per cent
2. Barred bacilli	Type C_1	.5	" "
3. Solid bacilli	Type A_2	2.6	" "
	Type C_2	9.7	" "
	Type D_2	3.2	" "

B. Tonsillar and Laryngeal Diphtheria		20 cases	
1. Granular bacilli	Types A, B, and C (mostly C)	80.0	per cent
2. Solid bacilli	Type A_2	2.0	" "
	Type C_2	10.0	" "
	Type D_2	8.0	" "
C. Laryngeal Diphtheria		39 cases	
1. Granular bacilli	Types A, B, and C (mostly C)	69.2	per cent
2. Solid bacilli	Type A_2	5.1	" "
	Type C_2	20.5	" "
	Type D_2	5.1	" "
D. Nasal Diphtheria		8 cases	
1. Granular bacilli	Mostly C	50.0	per cent
2. Solid bacilli	Type C_2	25.0	" "
	Type D_2	25.0	" "

The granular types of bacilli predominate in the largest proportion of cases of clinical diphtheria whether the disease is located in the nose, pharynx, or larynx. These types are likely to prove virulent even in prolonged culture cases and contacts, and should always be considered dangerous and positive until proved otherwise, regardless of the clinical aspect or history of the case.

Our experience with the barred types occurring early in diphtheria is quite limited. They are distinctly rare about Philadelphia, and are more likely to be found in cultures taken late in the disease. We consider them similar to the granular types, although the barred types are frequently harmless non-virulent bacilli and may be found in 20 per cent of cultures from the healthy penis.

The solid types give the most concern. We have often encountered cultures of extensive exudates in severe clinical diphtheria which showed a few scattered solid bacilli scattered among cocci or diplococci. Secondary cultures are usually of granular types. To disregard the few solid bacilli in the primary culture would frequently throw the clinician off guard and be the cause of much mischief. Many primary cultures from severe cases may be entirely free of diphtheria bacilli. These results are frequently due to faulty methods in making the culture, for if one is satisfied with a light perfunctory swabbing over the exudate, only the organisms of secondary infection may be secured and not the diphtheria bacilli deep in the exudate and near the living tissues. On the other hand, "carrier" cases are likely to show the presence of a solid type of bacillus. Nevertheless, these bacilli, A_2, B_2, and

C_2, should be held until subsequent cultures are secured. If the patient is entirely well and runs a prolonged course of positive cultures the bacilli should be tested for virulence, to avoid a needlessly prolonged quarantine.

The short, solid D_2 type of diphtheria bacillus resembles Hofmann's bacillus and is especially troublesome in nose cultures. Occasionally it is virulent and is the only bacillus to be found in a case of clinical diphtheria. It is especially found in "carrier" cases and is therefore frequently encountered in cultures from suspected "contacts." In the great majority of cases if two consecutive cultures of a person free from clinical evidences of diphtheria shows the presence of this organism the results may be considered negligible. This type of diphtheria bacillus should not be confounded with Hofmann's bacillus. Both may prove non-virulent in massive doses to a guinea-pig, but if acid is produced with any of the sugars the culture is a true D_2 type—a non-virulent diphtheria bacillus. If acids are not produced with the sugars it is likely to be Hofmann's bacillus.

As a general rule, we believe that the morphological classification of Wesbrook, Wilson, and McDaniel is good. The recognition of types requires experience and frequent checking up of opinions by isolating and studying cultures. The method has certain drawbacks and requires much care, but in routine work it is of distinct value. If one could always be guided by bacteriological and clinical findings in the management of diphtheria even better results would be secured. But clinical ability and opinions differ a great deal among practitioners and few are capable of co-relating the clinical and bacteriological data.

CHANGEABILITY OF TYPES OF BACILLI.

Another interesting feature in the bacteriology of diphtheria is the change in types during the course of the disease. Solid types of bacilli are found more frequently at the end of the course than at the beginning. Cobbett claims that solid types are usually present from the beginning but in the early cultures are overshadowed by the granular bacilli and subsequently when these disappear the solid types become prominent.

In the 253 cases referred to above the culture records show the following results:

1. Granular types persisting throughout	55.4	per cent
2. Solid types persisting throughout	15.2	" "
3. Granular types replaced by solid types	24.7	" "
4. Solid types replaced by granular types	4.5	" "

It will be noted that in about 70 per cent of cultures the bacilli adhere to their group characteristics. We believe with Cobbett that the transformation of types is in most cases only apparent. A study of cultures carried through pigs and subcultured over a period of time shows that while the types of a group may change, as A into C, or a C_2 type become shorter and resemble D_2, yet the complete transformation of a granular bacillus into a solid, or vice versa, is decidedly uncommon. Not infrequently, however, a culture of D_2 may be found to show granular types after longer incubation, especially in cultures from clinical diphtheria. The replacement of solid by granular types often means reinfection of the patient, with the formation of a pseudo-membrane and the general symptoms of diphtheria.

ANIMAL INOCULATION TESTS.

Loeffler originally found that virulent diphtheria bacilli produce more or less characteristic changes when injected into a guinea-pig, prominent among which is the development of gelatinous edema at the site of injection. Since then abundant evidence has accumulated in support of his findings, and with a suitable technic the virulence of a given culture is readily determined. A question of greater importance, however, is whether an organism found non-virulent for the guinea-pig may regain its virulence when transferred to human tissues. In most cases it is comparatively simple to restore virulence to a culture by repeated "passage" through animals, hence the question regarding the delicacy of the pig-test for virulence. Are the tissues of the animal delicate enough to react to the influence of a slightly virulent culture? If a given culture were found to produce no disturbances in a pig and yet produce diphtheria in the human being, then the inoculation test

as a routine procedure is a mistake, and dangerous on account of its misleading information.

The practical benefits of such tests may be considerable because in this manner a quarantine may be lifted from a person or family suffering hardship by reason of prolonged isolation and detention. The attending physician may regard lengthy quarantine as a reflection upon his ability and a reversal of his opinion regarding the condition of his patient, and he may in this manner suffer in professional reputation. This, leading to antagonism between public officials and practitioners, is detrimental to public health, for without the hearty co-operation of the latter little real good can be accomplished.

It is understood, therefore, that while inoculation tests are often of much aid in the practical management of diphtheria they are, at least when conducted on a large scale, expensive, laborious, and time consuming. In an experimental way they are of much value in differentiating between true diphtheria bacilli and Hofmann's bacillus when used in conjunction with acid-production tests.

On the other hand, the differentiation between dangerous, doubtful, and harmless diphtheria bacilli according to their morphology is quick, comparatively easy, and inexpensive. Such a method has technical difficulties, as already dwelt upon, and is dangerous in the hands of the inexperienced. Nevertheless, experience has taught that such a method has distinct value, and when used together with judicious animal inoculation tests a highly satisfactory technic results.

Many observers have noted that diphtheria bacilli retain their virulence until they disappear, regardless of the length of their stay. Judging from our own results, we cannot agree entirely with this view. After a time, the minimum being not less than two weeks, a proportion of bacilli prove non-virulent when tested by animal inoculation. Whether or not they regain virulence when passed from throat to throat is the important question, difficult to answer because the disease may be spread in such a varied and confusing manner. We have no direct evidence, however, that this occurs. Most physicians depend upon two consecutive nega-

tive cultures before discharging a patient, but when one stops to consider the fallacies of such a method and the goodly proportion of those who will yield a positive culture if cultured the third time, showing thereby that they are not really free of the bacilli, it is a question whether or not a good pig-test is not to be preferred.

We believe that in some instances the prolonged number of positive cultures following diphtheria are due to the presence of harmless diphtheria-like bacilli of "carriers," such as are found in about 12 per cent of healthy throats and about 20 per cent of healthy noses, and having no connection with clinical diphtheria. They were present before the attack of diphtheria and are likely to remain for an indefinite time afterward. At first they are overgrown by the disease-producing organisms, but as these disappear the "mutants" become prominent again and yield a formidable line of positive cultures. A pig-test is certainly justifiable in cleaning up such a case.

If the animal inoculation test is to be accepted as an indication of the virulence or non-virulences of a given bacillus the technic must be satisfactory. Almost any procedure suffices when dealing with highly virulent bacilli, providing the bacilli are injected into the pig. But when dealing with bacilli of decreased virulence certain steps in technic become important. With a slightly virulent culture it is easily demonstrated that results vary according to differences in technic. The most important factor in inoculation work is the detection of slightly virulent cultures, because it has been abundantly proved that these may become fully virulent by "passage" through animals and probably also by transfer from throat to throat.

It should also be emphasized that in the same culture tube colonies of virulent and non-virulent bacilli may be growing side by side, and if a single colony is chosen for growing the culture for injection in conducting a pig-test the non-virulent may be picked out. To choose many colonies and test each separately requires many animals and becomes expensive. This difficulty may be obviated somewhat by inoculating a tube of broth with many different colonies. If sufficient time is permitted for growth the virulent bacilli will multiply to such an extent as to make their presence known.

The following 237 animal inoculation tests were conducted routinely in the Laboratory of the Philadelphia Hospital for Contagious Diseases with cultures from patients who had recovered from diphtheria and were running positive cultures. It also includes a smaller number of "carrier" cases, yielding positive cultures of diphtheria bacilli but presenting no clinical evidences of disease. From time to time a pig-test, not included here, was conducted with cultures of different types of bacilli from clinical cases, as controls over the technic. The results of these are considered in the conclusions bearing upon this subject.

TECHNIC.

a) *Types of bacilli.*—For the sake of brevity all the types found in the cultures are not recorded. In a given culture of granular bacilli types A, B, and C may be found, although if one predominates, the culture is recorded according to that type. The greater proportion of granular bacilli are of those three types and seem to be of equal virulence. We rarely find a true banded bacillus and have no record of a pig-test with bacilli of that type. Considerable difficulty frequently occurs in drawing sharp lines among the solid bacilli. If the temperature of the incubator goes up over night the bacilli are found short next morning and as a result a different type may be recorded. The A_2 type is usually easy of detection; to differentiate between B_2 and C_2 and between D_2 and E_2 is frequently most difficult. Most of our long solid types we have classified as C_2 and the short types, D_2. The cultures for the pig-test were recorded as follows:

A. Granular bacilli, A, B, C, and D—mostly C.
B. Solid bacilli:
    Long solid—A_2, B_2, and C_2—mostly C_2.
    Short solid—D_2 and E_2—mostly D_2.

b) *Animal inoculation test.*—

1. Isolation by "streak" method.

2. Examination of colonies; inoculation of a tube, containing 6 to 8 c.c. of neutral or slightly alkaline broth containing 1 per cent glucose, with several different colonies.

3. Broth culture slanted and grown for 72 hours at 35° C.

4. Examination of broth cultures for purity of growth. A healthy guinea-pig weighing between 250 and 300 gms. is injected in the median abdominal line with a dose of culture corresponding to 0.5 per cent of the body weight of the animal expressed in cubic centimeters. Thus a 260 gm. pig receives 1.3 c.c. of culture.

5. The animal is kept under observation for at least four days and examined for edema at the site of injection and for evidences of toxemia.

6. In case of death autopsy is performed and cultures made from site of injection and various internal organs, including the peritoneal cavity. The test is regarded as positive and a second pig injected with toxin-antitoxin mixture to determine more conclusively if death was due to diphtheria bacilli.

Certain points in technic require special mention. The reaction of the broth should be neutral or slightly alkaline because diphtheria bacilli produce acid rapidly, especially with glucose, and high acidity inhibits and finally kills the bacilli. Glucose aids in growing richer cultures. Inoculation of broth from solid serum media may require previous "education" by several daily subcultures before a good broth growth is secured. Animals over 300 gms. are too resistant to diphtheria to be safe for inoculation work. We feel reasonably certain that when the bacilli have once been "educated" to grow in broth sufficient toxins are elaborated in 48 hours to produce a positive result when injected in the dose recommended. For fear of missing a slightly virulent culture, however, we have extended the time by 24 hours. This extension does not make any material difference, because it takes at least a week to conduct the test under the best of circumstances. A toxic animal is regarded as a positive result, even though it does not succumb, until proved otherwise by toxin-antitoxin injection.

c) *Results.*—

A. Cultures from Throat			91
1. Granular types (mostly C)			60
Positive	63.2	per cent	
Negative	36.6	" "	
2. Solid types (mostly C_2)			23
Positive	26.0	" "	
Negative	74.0	" "	

3. Solid types (mostly D_2)		8
Positive	12.5 " "	
Negative	87.5 " "	
B. Cultures from Nose		68
1. Granular types (mostly C)		25
Positive	56.0 per cent	
Negative	44.0 " "	
2. Solid types (mostly C_2)		23
Positive	26.0 per cent	
Negative	74.0 ". "	
3. Solid types (mostly D_2)		20
Positive	15.0 per cent	
Negative	85.0 " "	
C. Cultures from Ears		78
1. Granular types (mostly C)		27
Positive	47.0 per cent	
Negative	53.0 " "	
2. Solid types (mostly C_2)		24
Positive	8.0 per cent	
Negative	92.0 " "	
3. Solid types (mostly D_2)		27
Positive	6.0 per cent	
Negative	94.0 " "	

d) Conclusions.—

1. The granular types of bacilli gave the highest percentage of positive tests. In clinical diphtheria they are almost invariably virulent for guinea-pigs. No matter how long they persist in the throat after recovery they should be regarded as dangerous until proved otherwise. Of all types they are most likely to retain their virulence.

2. The value of animal inoculation tests is especially appreciable when dealing with solid types of bacilli. The longer types are more likely to retain virulence than the shorter varieties. Both, however, may be descendants of the "carrier" bacillus rather than of the bacillus producing the clinical evidences of the disease.

3. The short solid types of bacilli are especially common in the nose in the absence of clinical diphtheria. In a small percentage of cases these bacilli are truly pathogenic but the majority of cultures are found without virulence when tested by animal inoculation.

4. The long solid types of bacilli, so frequently found in otitis media, are in the majority of cases non-virulent. We have tested

over 30 cultures of these bacilli isolated from cases of suppurative otitis media (not included in this series) with uniformly negative results.

5. Animal inoculation tests have a special field of usefulness in testing the solid types for virulence when they persist in the tissues over a long period of time or when found in contact and "carrier" cases.

ACID-PRODUCTION TESTS.

Differentiation among members of the diphtheria group of bacilli has been studied by means of acid-production tests, the bacilli differing in their power to break up certain sugars with the formation of acids. Such tests have been advocated as a means of studying and classifying the Hofmann bacillus and *B. xerosis*.

We have tested 62 cultures of various types of bacilli from clinical cases of diphtheria of the eye, ear, nose, and throat, and also from "carrier'" cases, according to the following technic:

1. Hiss's serum-water media were used, colored with azolitmin for indicator and containing, respectively, 1 per cent of the following sugars: glucose, saccharose, dextrin, lactose, maltose, galactose, and mannite. The purest products only should be used. The lactose media frequently become acid and give trouble. Non-inoculated controls were always incubated along with the cultures.

2. Old cultures were "educated" to grow in the new media by two or more daily subinoculations.

3. Results were recorded after cultures had been incubated for five days at 35° C. The presence of acid was shown by the change in color—the blue being turned to pink or red.

The titration of total acidity in glucose broth cultures with sodium hydrate, with phenolphthalein as indicator, yields interesting and more important results, especially when studying the Hofmann bacillus.

The results of the work may be expressed as follows:

1. Virulent diphtheria bacilli produced acid most frequently with dextrose, next in frequency with dextrin. With the remaining sugars the results varied considerably.

2. Cultures proving non-virulent and producing acid with

some of the sugars, especially glucose and dextrin, were regarded as non-virulent diphtheria bacilli.

3. The sugar tests were found of most value in studying cultures from the eye and in dealing with D_2 types. Short, solid types proving non-virulent for pigs and producing no acid with any of the sugars were regarded as cultures of Hofmann's bacillus. Non-virulent cultures producing acid with saccharose were regarded as cultures of *B. xerosis*.

4. In routine work the sugar-tests are of distinct value when used in conjunction with tests for virulence. The short, solid D_2 type should not be confounded with Hofmann's bacillus, and since both may be non-virulent the sugar-tests alone can differentiate.

DISCUSSION.

Although great advances have been made in the diagnosis and treatment of diphtheria, yet the fact remains that the disease occurs with almost unabated frequency. Before the morbidity can be effectually lowered there must be hearty co-operation between practitioners and public health officials. There will always be the atypical and unrecognized cases to be dealt with—cases capable of spreading the disease—but with the conscientious use of the culture outfit a number of these may be recognized. The ability of the average practitioner to diagnose diphtheria in its various manifestations, and especially to differentiate the infection from similar clinical conditions, is as a rule overrated. Many insist upon the typical test-book picture of diphtheria before making a diagnosis, others depend too much upon the results of bacteriological examination. Diphtheria of the nose is especially likely to be confusing.

The physician should realize his limitations in making correct differential diagnoses and properly culture all "suspicious" cases. He should be thoroughly acquainted with the etiology of diphtheria and recognize the existence of "carriers" of dangerous bacilli and the possibility of others contracting the disease. He should know likewise that virulent bacilli may persist in the tissues of his patient for a long time although the latter feels and looks perfectly well. And furthermore he should take pains to explain these

factors to his patient or the family in order to protect himself in case a prolonged quarantine is necessary. Too many practitioners commit themselves to a diagnosis in a doubtful case before the results of cultures are ascertained, and consequently are often required to change their diagnosis. Others promise quick recovery and relief from quarantine when they should know they are unable to foretell the length of quarantine if such is to be regulated by culture.

One negative culture from a throat showing extensive exudate should not be accepted as evidence of absence of diphtheria. Clinical judgment should rule and antitoxin be administered. A second culture should then be made. No medical man is justified in withholding antitoxin 24 hours to await the report of a culture. The fear of anaphylaxis in case the patient has received serum on a previous occasion is overdrawn and too prominent in the minds of medical men. Cultures should be taken, and taken repeatedly, from persons in contact with the patient, if sore throat or nasal discharge develops. A hospital ward offers the greatest practical difficulty because where a large number are cultured, a few are most likely to show the presence of diphtheria-like bacilli which may or may not be virulent. Such persons should be removed for a day or so until further bacteriological examinations are made. In the meantime a sharp lookout is maintained for clinical developments. The wholesale taking of cultures, especially of those not in immediate relation with the patient, is unnecessary.

For the proper management of diphtheria according to bacteriological examinations an effort must be made to differentiate between the harmful and harmless bacilli. For instance, a "carrier" may be quarantined forever if guided by the fact that the presence of a diphtheria-like bacillus means diphtheria. The medical attendant must be convinced of the value of bacteriological examinations and of the earnest efforts of the bacteriologist to work with him both in the interests of his patient and for the public welfare. The practitioner must be taught not to be afraid to report all of his cases and to culture faithfully. If, however, bacteriological examinations serve to give unnecessary embarrassment to the practitioner by quarantining "carrier" cases, or by

maintaining a quarantine unnecessarily long after convalescence as a result of conscientious efforts on the part of the latter to do his duty in taking cultures, he is not likely to give his best co-operation, and without this no real good is possible.

We advise the following scheme, modified after Wesbrook, of bacteriological diagnosis and management of diphtheria:

1. Hold the granular types, A, B, C, D, and E, as significant, regardless of the clinical condition or history of the patient or of the presence or absence of other types. Cultures of these types from "carriers" or from persons running a prolonged list of positive cultures should be tested for virulence.

2. Regard the barred types, A_1, B_1, C_1, and D_1, as "doubtful" unless granular bacilli are also present when a positive diagnosis is given.

3. Hold the solid types, A_2, B_2, C_2, and D_2, as "doubtful" and request another culture. If granular bacilli are present a positive diagnosis is given. If subsequent cultures show the same solid types and if the patient is free of clinical evidences of diphtheria they may be regarded as negligible. It is in dealing with these types that we feel the greatest need for clinical co-operation, because clinical diphtheria may in exceptional instances be caused by these bacilli, although in the majority of cases the D_2 type is a negligible organism.

4. Regard E_1, E_2, F, G, F_1, and F_2, as negligible.

5. Test the virulence of all cultures of solid types of bacilli after the patient has recovered and is being quarantined over a long period of time by positive cultures. These inoculation tests are especially indicated with cultures from the nose and ear.

If the above method is employed by the bacteriologist and if three successive negative cultures are required instead of two before quarantine is lifted, combined with the judicious use of the animal inoculation test in suitable cases, we feel that the bacteriological diagnosis and management of diphtheria will be efficient and satisfactory to all.